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(54) Title: SACCHARIDES, THEIR SYNTHESIS AND USE

(57) Abstract

A library comprises at least six different sugar-containing molecules selected from (i) carbohydrates each derived from at least two saccharide monomers, and (ii) glycoconjugates. Such a library may comprise disaccharides or linear or branched trisaccharides. It may be prepared from a compound having at least two OH groups which are modified by independently-removable blocking groups. A novel process comprises synthesising a polysaccharide of the formula $sugar_n$, n representing the number of mono- and/or oligo-saccharide units from which the polysaccharide may be derived, comprises the steps of coupling $sugar_2$ with a conjugate of the formula Q-$sugar_1$, Q being a removable lipophilic or polymeric material, in a solvent for the conjugate; adding a non-solvent for the resultant conjugate Q-$sugar_1$-$sugar_2$; repeating the said steps n-2 times, as necessary, selecting the sugar to be coupled independently each time; and removing Q from the resultant coupled conjugate.

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SACCHARIDES, THEIR SYNTHESIS AND USE

Field of the Invention

This invention relates to mixtures of carbohydrates, their preparation and use. This invention relates also to multi-functional compounds, the use of such compounds to prepare carbohydrate conjugates, the conjugates themselves and also their use, e.g. in therapy and as addressable libraries.

Background of the Invention

Peptide libraries, i.e. mixtures of a large number of peptides, are well known and widely used, e.g. for screening for drugs and antibodies. For a review of peptide synthesis, including solid-phase synthesis methods, and peptide libraries, see Jung et al, Angewandte Chemie 31(4):367-383 (April 1992), and Birnbaum et al, Current Opinion in Biotech. 3:49 (1992).

Glycoamino-acids are known and have been used in the synthesis of glycopeptides. Solution-phase synthesis is described by Kunz, ACIE 26:294 (1987). Solid-phase methods are described by Chadwick et al, Biochem. Soc. Trans. 19:4075 (1991), and by Otvos et al, Tet. Lett. 31:5889 (1990), and Pept. Res. 2:362 (1989).

Kahne et al describe mixtures of carbohydrates with a limited number of repeating units, in unequal proportions.

Various solid-phase saccharide syntheses have been proposed. By coupling an acceptor sugar to a stationary phase, and conducting the sequential saccharide coupling reactions, to obtain an immobilised polysaccharide, the various reactants and any by-products can be removed from the system before the desired polysaccharide is removed from the support.

Solid-phase oligosaccharide synthesis is potentially rapid and efficient, as has been found for the now widely-used solid-phase syntheses of polypeptides and polynucleotides. However, coupling efficiency is generally low, and stereospecificity is often lost during glycosidation, as discussed by Malik et al, Chemiker-Zeitung 114 (12):371-375 (1990).

Oligosaccharide synthesis in solution is also known, and allows anomeric control. Howev r, it is insufficiently rapid for commercial exploitation. Semi-solid phase synthesis has been proposed, but it is difficult to make the PEG-acceptor sugar conjugate available for coupling at the anomeric position, and cleaving the desired polysaccharide from the PEG often cannot be conducted under convenient conditions.

Glycoconjugates, comprising carbohydrates linked to polymers, e.g. via ester or thio groups, are known. See Douglas et al, JACS 113:5095-7 (1991).

Summary of the Invention

One aspect of the present invention is based on a realisation of the utility of carbohydrate-type libraries. For clear distinction from mixtures of carbohydrates or glycoconjugates that may have been prepared, with or without intent, in the past, such libraries comprise at least 6 different carbohydrates or glycoconjugates, although the number may be much higher, e.g. a minimum of 10, 20, 30, 40, 50, e.g. up to 100, or higher. The library can be used for efficient mass screening of large numbers of carbohydrates or glycoconjugates, for biological or any other activity.

In another aspect, the present invention is based on the realisation that a monosaccharide or other polyhydroxy compound has a variety of different reactive groups which can be used in order to obtain conjugates having a combination of functional moieties in any desired combination of stereochemical or other relationships. For this purpose, a compound of the invention comprises 2, 3, 4, 5 or more independently removable blocking groups. For selective reaction to form a conjugate, one of the blocking groups may be removed in the same or a separate step.

For the avoidance of doubt, there may one or more of any of the blocking groups. Thus, for example, there may be a first group removable under first conditions, a second group removable under second conditions, and two third groups removable under third conditions.

According to a further aspect of the invention, a process for synthesising a polysaccharide of the formula $sugar_1$-$sugar_2$ comprises coupling $sugar_2$ with a conjugate of the formula Q-$sugar_1$, Q being a removable lipophilic or polymeric material, in a solvent for the conjugate; adding a non-solvent for the resultant conjugate Q-$sugar_1$-$sugar_2$, and removing Q from the resultant coupled conjugate. This is based on the discovery that Q can be chosen so that the conjugate is soluble, but can simply be rendered non-soluble in the system, allowing ease of separation, and also on the discovery of materials Q that are easily separated from the desired conjugate.

If desired, in this aspect of the invention, the steps recited above may be repeated n-2 times, as necessary, selecting the sugar to be coupled independently each time, to prepare a polysaccharide of the formula $sugar_n$. n represents the number of mono- and/or oligo-saccharide units from which the polysaccharide is derived. $Sugar_1$ will usually be a monosaccharide; monosaccharides may be used sequentially linked to the sugar-conjugate, but di- and higher saccharides may be coupled in a single step.

Description of the Invention

In one presently preferred embodiment of the invention, carbohydrate libraries, consisting of di- and trisaccharides, are developed in a fashion formally analogous to the strategy used to develop peptide libraries. Each carbohydrate library consists of multiple compounds, some or all of which may be known. Multiple such libraries may be prepared. Each library can be prepared in such a way as to be fully "addressable", i.e. so as to allow subsequent "decoding" through synthesis of restricted sub-libraries and, if appropriate, chromatographic or other fractionation. This allows the identity of any individual carbohydrate in an active library to be readily addressed.

Using suitable procedures, examples of which are known to the skilled man, the saccharides can be prepared free of metal ions, solvents and extraneous cytotoxic or other interfering substances, for use in screening assays. For

example, many saccharide coupling reactions and conditions are known. Solid-phase syntheses are known (s above); a semi-solid phase synthesis that is the third aspect of this invention is described in more detail below.

There are significant advantages in being able to perform carbohydrate library synthesis using solid-phase reaction procedures. The principal advantages are more rapid separation of products and reactants, and the ability to increase significantly the molecular diversity, by allowing additional reactions and extensions. Given for the purpose of illustrating the present invention, the following description is premised on solution-phase synthesis. Solution-phase synthesis of carbohydrates is considered completely practical since, even for a trisaccharide library, only two covalent bonds are being formed.

It is preferred that the different compounds in th library are in equimolar proportions. Depending on the reactants and conditions used, precise equimolarity may not be realistic, although it is desirable to aim for maximum acceptor reactivity.

In preparing the library, it is certainly generally preferred that equimolar amounts of differentially-protected acceptors be used. This mixture is then reacted with differentially-protected donors. Protected compounds which can be used as acceptors and donors include the second aspect of this invention.

More generally, the polyhydroxy compound that is used as a "scaffold" for the purposes of this invention may be any known, say, steroid or sugar molecule, preferably a di- or trihydroxy compound such as a monosaccharide or, for example, cholic acid methyl ester. Each such molecule has a number of different active groups, and blocking groups that can be introduced and removed selectively, as desired, e.g. sequentially, and the means for conducting these processes, are well known.

If the "scaffold" is a saccharide, first, second and further moieties may be coupled with a compound of the inv ntion in known manner. Various coupling techniques

suitable for saccharides, to form glycoconjugates, are known.

A compound of the invention may also be used to generate a library of conjugates that is fully addressable, as described above. By way of illustration, in order t prepare a chiral template-based/mixed species library, syntheses are conducted, starting with a trihydroxy compound that may be known per se, to prepare a derivative having combinations of substituents OX, OY and OZ. For example, the following 6 possible combinations of a compound I may be generated, e.g. with the following substitution profile:

X = H Y = Ac Z = Bn
X = H Y = Bn Z = Ac
Y = H X = Ac Z = Bn etc..

An equimolar mixture of the 6 versions of A is divided into 3 equal parts. One part is then reacted with a new reagent R_1, the second part with a reagent R_2 and the final part with R_3. The compounds R may be, for example, glycosyl donors, activated lipophiles, phosphorylating agents or a variety of other compounds.

The 3 parts are mixed after the reaction has gone to completion, then worked-up and de-O-acetylated to afford a mixture of 18 different compounds. The mixture is then re-divided into 3 parts, one of which is reacted with reagent R_4, one with R_5 and one with R_6. Following completion of the reactions, the parts are mixed and worked-up to give a protected library consisting of 54 compounds.

The procedure may be extended one more time, making use of the third hydroxy function which is first deprotected (debenzylation). The steps of re-division, separate reactions and re-combination are carried through just as before so that after any required final deprotection steps a library of 162 components is obtained.

The same procedure may be adopted starting with a dihydroxy compound. In this case, at least one OH group is blocked.

Glycosylation with a particular differentially-protected monosaccharide donor thus gives a library of disaccharides. For libraries of larger molecules, selective deprotection and subdivision, and then further coupling, may be repeated, as desired.

Another presently preferred embodiment of the invention lies in glycoconjugate libraries, consisting of conjugated mono-, di- or tri- saccharides, that may also be developed in a fashion analogous to the strategy used to develop peptide libraries. The characteristics and preparation of such libraries are broadly analogous to those described above for carbohydrate libraries. For example, the acceptor may itself be a glycoconjugate, including one saccharide molecule, to which a second and, if desired, further mono-saccharides are conjugated, by th procedures generally described herein. Alternatively, the acceptor may be a mono-saccharide, di-saccharide or higher holo-saccharide, in which case the donor provides a non-saccharide function. Specific examples of donors are acidic functions such as sulphonate and phosphonate groups, or a mixture of such groups. By way of illustration, a sulphonate group may be introduced by reacting the acceptor with excess chlorosulfonic acid.

The glycoconjugate may be, for example, a glycopeptide. Glycoamino-acids may be used to prepare glycopeptide libraries, essentially by using them (in suitably protected and activated forms) in place of one or more of the amino-acids used to prepare peptides libraries by known methodology. The term "glycoamino-acid" refers to any natural or unnatural or modified amino-acid coupled to any natural or unnatural or modified sugar (with one or more mono-saccharide residues). The sugar moiety and the amino-acid moiety may be linked via an oxygen atom, as in tetra-O-acetyl Glucose-β-Fmoc-serene, or via a nitrogen atom, as in tri-O-acetyl GlnNa-β-Fmoc-Asn. Other examples of glycoamino-acids are shown below, as formulae IIA to IIF. These compounds are resp ctiv ly

Tri-O-acetyl xylose-β Fmoc threonine

Tetra-O-acetyl-glucose-β Fmoc hydroxyproline

Tetra-O-acetyl-glucose-β Fmoc serine
Tri-O-acetyl GlCNAc-β Fmoc Asn
Tetra-O-acetyl glucose-β Fmoc Asn
Tetra-O-acetyl glucose-α Fmoc Asn

Further by way of illustration, the invention will now be described by way of example only with reference to specific experimental procedures that may be used to obtain libraries of synthetic carbohydrates.

The coupling gives mixtures that are distinguished from random mixtures by being deliberately controlled and designed, without restriction to means. That means may be, for example, enzymatic or chemical (using excess donor).

Available Monosaccharides: The range of pure monosaccharides available commercially or through very simple synthesis in large amounts (multi-gram to kilogram) is quite large. For the purpose of this invention, and to simplify arithmetic, discussion will be confined to the following twelve monosaccharides. It must be remembered that a significantly larger repertoire of monosaccharides could in fact be used, encompassing fluorinated monosaccharides, de-oxy monosaccharides, and all manner of sugar-based structures.

Group I Monosaccharides (Tetra-hydroxy)	Group II Monosaccharides (Penta-hydroxy)
A = L-Fucose	G = D-Glucose
B = D-Xylose	H = D-Galactose
C = L-Rhamnose	I = D-Mannose
D = D-Arabinose	J = D-Allose
E = D-GlcNAc	K = D-Talose
F = D-GalNAc	L = L-Idose

Forms of Libraries: Constraining the number of monosaccharides in a library to be ≤3, so as to satisfy the requirement for MW ≤500 for each compound in a library, thr e kinds of carbohydrate library can be envisaged. They are:

disaccharide library : Donor - Acceptor

linear tri-saccharide library : Donor - Donor - Acceptor
branched tri-saccharide library : Donor - Acc ptor - Donor

Monosaccharide Acceptors and Donors: Due to the chirality and poly-hydroxy nature of each monosaccharide, several different acceptor and donor forms exist for each monosaccharide for the purpose of chemical conjugation. This property of monosaccharides is uniquely attractive from the point of view of library synthesis, since it allows a relatively large number of individual compounds to be synthesised from relatively few monosaccharides, and with correspondingly low molecular weight.

In donor mode, each monosaccharide may be activated at C-1 by means of a thiomethyl activating group and O-benzyl at C-2 (to favour α-anomeric linkage) or O-acetyl at C-2 to form the ß-anomeric linkage. The protection of the hydroxyl functions at C-3, 4 and 6 may depend on whether a synthetic di-saccharide, linear tri-saccharide, or branched tri-saccharides library is being prepared. In the case of a di-saccharide library, or a library of branched tri-saccharide, or the second donor in a library of linear tri-saccharide, all hydroxyl functions (other than C-1) may be similarly protected. In the case of a library of linear tri-saccharides, for the first donor, one hydroxyl group in the ring may would be acetylated and all others benzylated. This allows specific selective deprotection after the first conjugation, so as to allow subsequent conjugation of the second donor. For example, the donor to form the terminal ß-linked D-galactose linkage in a di-saccharide library, or one of branched tri-saccharides, or the second donor in a library of linear tri-saccharides, is of formula III.

For each of the twelve exemplified monosaccharides, benzylated thiomethyl derivatives can be easily prepared, as can fully acetylated ones. For the purpose of calculation, it is assumed that twenty donors can be generated from the twelve monosaccharides.

The first donor during preparation f a library of linear tri-saccharides to form a glucose linkage may be any of formula IV, wherein one of W, X, Y, and Z is Ac and the

other 3 groups are each Bn. Again for the purpose of calculation, it is assumed that forty-two donors of this form can be generated from the twelve example monosaccharides.

The repertoire of donors can therefore be summarised as follows:

Form of Library	Number of First Donors	Number of Second Donors
Di-saccharide	20	-
Linear tri-saccharide	42	20
Branched tri-saccharide	12	20

In acceptor mode, each monosaccharide may be protected at C-1 by benzylation of the C-1 hydroxyl function. Protection of the hydroxyl functions at C-2, 3, 4, and 6 may depend on the form of library that is being prepared, namely di-saccharide, linear tri-saccharide, or branched tri-saccharide. In the case of di-saccharide and linear tri-saccharide libraries, one of the available ring hydroxyl positions may be available and the others protected. For example, the N-acetyl-D-glucosamine acceptors for these two kinds of libraries is formula V, wherein one of X, Y and Z is H, another is Ac and the third is Bn.

In the case of a library of branched tri-saccharides, one hydroxyl function may be left available, on acetylated, and the others benzylated. For example, the corresponding N-acetyl-D-glucosamine acceptors for this form of library are of formula VI, wherein one of X, Y and Z is H, another is Ac and the third is Bn.

The repertoire of acceptors can therefore be summarised as follows:

Form of Library	Number of Acceptors
Di-saccharide	42
Linear tri-saccharide	42
Branched tri-saccharide	108

The repertoire of donors and acceptors for the three forms of libraries using the twelve monosaccharides listed earlier by way of example, can be summarised as follows:

Library	No. of Acceptors	No. of First Donors	No. of Second Donors
Di-saccharide	42	20	-
Linear tri-saccharide	42	42	20
Branched tri-saccharide	108	12	20

All the above listed forms of donors and acceptors can be prepared by the skilled man.

Preparation of Di-saccharide Libraries

Step 1: An equimolar mixture of the 42 acceptor monosaccharides is prepared.

Step 2: This mixture is divided into 20 equal aliquots.

Step 3: Each aliquot is fully glycosylated with a single donor in large excess.

Step 4: Conjugated acceptors are separated from unreacted donor by chromatography.

Step 5: The di-saccharide library is fully deprotected.

In this way 20 libraries are prepared, with each library consisting of an equimolar consisting of an equimolar amount of each of 42 di-saccharides.

Preparation of Linear Tri-saccharide Libraries

Step 1: An equimolar mixture of the 42 acceptor monosaccharides is prepared.

Step 2: This mixture of acceptor monosaccharides is divided into N_1 equal aliquots where $N_1 \leq 42$.

Step 3: Each aliquot is fully glycosylated with a single donor, of the form of the first donor for th linear tri-saccharide library. There are 42 such donors.

Step 4: Conjugated acceptors are separated from unreacted first donor by chromatography.

Step 5: Each di-saccharide library is de-O-acetylated and divided into 20 equal aliquots.

Step 6: Each aliquot is fully conjugated with a single donor, of the form of the second donor for th

lin ar tri-saccharide library. There are 20 such donors.

Step 7: Conjugated acceptors are separated from unreacted second donor by chromatography.

Step 8: The linear tri-saccharide library is fully deprotected.

Using this approach, two extreme cases can be envisaged. In one case, there are 20 libraries, each containing an equimolar mixture of 42 x 42 = 1764 linear tri-saccharides is prepared. In the other case, 840 libraries are prepared, each containing an equimolar mixture of 42 linear tri-saccharides. Any combination of number of libraries and number of compounds per library could be prepared, in a fully addressable way, by varying N_1.

Preparation of Branched Tri-saccharide Libraries

Step 1: An equimolar mixture of the 108 acceptor monosaccharides is prepared.

Step 2: This mixture of acceptor monosaccharides is divided into 12 equal aliquots.

Step 3: Each aliquot is fully glycosylated with a single donor, of which there are 12 different ones. This generates 12 libraries of 108 compounds in each.

Step 4: Conjugated acceptors are separated from unreacted first donor by chromatography.

Step 5: Each library is de-O-acetylated and divided into 20 equal aliquots.

Step 6: Each aliquot is fully conjugated with a single donor, of which there are 20 different ones.

Step 7: Conjugated acceptors are separated from unreacted second donor by chromatography.

Step 8: The branched tri-saccharide library is fully deprotected.

In this way 240 libraries, each containing an equimolar mixture of 108 branched tri-saccharides, is prepared.

The repertoire of libraries and different carbohydrates per library can be summarised as follows:

Form of Library	No. Libraries	No. Carbohydrates per Library
Di-mers	20	42
Linear tri-mers	20	1764
Branched tri-mers	240	108

In addition to the basic libraries, further diversity may be incorporated, while still retaining equimolarity of individual carbohydrates. This may be achieved, for example, by

(1) increasing the repertoire of starting monosaccharide;

(2) using modified monosaccharide as starting sources e.g. fluorinated derivatives;

(3) tapping off intermediate di-saccharide mixtures formed during synthesis of tri-saccharide libraries, and using other chemical entities (such as sulphate, phosphate, or various organic groups) as the second donors.

In using the second aspect of this invention, the moieties that are introduced may be chosen because of their various biological or physical properties. One or more may have, for example, therapeutic or diagnostic utility, and may be chosen on the basis of such utility. In this case, the resulting conjugate according to the invention is suitable for therapeutic use.

The following Reaction Scheme XI → XII → XIII → XIV illustrates a differentially-blocked saccharide of the invention, and its use, to prepare a compound having alkyl, R_{11} and R_{12} substituents. The specific differentially-blocked monosaccharide that is shown is (1,4 - di-O-methyl, 3-O-benzyl, 6-O-benzyl, 6-O-tert-butyldimethylsilyl) Galactose. Three reactions with bromides, i.e. octyl bromide, $R_{11}Br$ and $R_{12}Br$, follow: each is conducted in the presenc of a silver salt activator.

The initial reaction on the differentially-protected monosaccharide is to put on an alkyl chain, perhaps to

enhance the oral bio-availability of the final product. Following deprotection of the 3-position, the next reaction is to add a grouping R_{11} based on AZT.

After deprotection of the 6-position, a function R_{12} is added which is based for example on the anti-infective agent Flagyl.

In this way, a structure can be built up which includes two groups having separate therapeutic effects and one group which modifies the bio-availability profile of the structure.

The third aspect of this invention is a process that is to give oligosaccharides by sequential coupling reactions. This process is illustrated in the Reaction Scheme XXI ($sugar_1$) + XXII ($sugar_2$) → XXIII → XXIV + XXV ($sugar_3$) → etc, where two differentially-protected molecules ($sugar_1$ and $sugar_2$) and a coupling agent are reacted in the first step, the second step involves deprotection, the third coupling etc.. After coupling, a non-solvent is added to cause precipitation, filtration removes deprotecting agent, and solvent is added for redissolution.

For example, the coupling is carried out in a solvent such as dichloromethane or acetonitrile; there are many alternatives, in which Q-$sugar_1$ is soluble as is $sugar_2$. After coupling, a non-solvent such as ether (again there are alternatives) is added such that, in the resulting solvent mixture, Q-$sugar_1$-$sugar_2$ is insoluble but $sugar_2$ and coupling agent are still soluble. The required, insoluble coupled material may therefore be filtered off, thus separating it from excess $sugar_2$ and coupling agent. Then it is redissolved in solvent, deprotected, reprecipitated and filtered from deprotecting agent. Finally, it is redissolved in solvent, and $sugar_3$ coupling agent added to continue the cycle.

The process of the invention is essentially a semi-solid phase synthesis. The support shown in the reaction schem is modified PEG. The modification involves the use of a benzyl linker group, thereby retaining the desired characteristics of PEG while facilitating removal of the

ultimate desired polysaccharide, by hydrogenation. An alternative is DDQ oxidation.

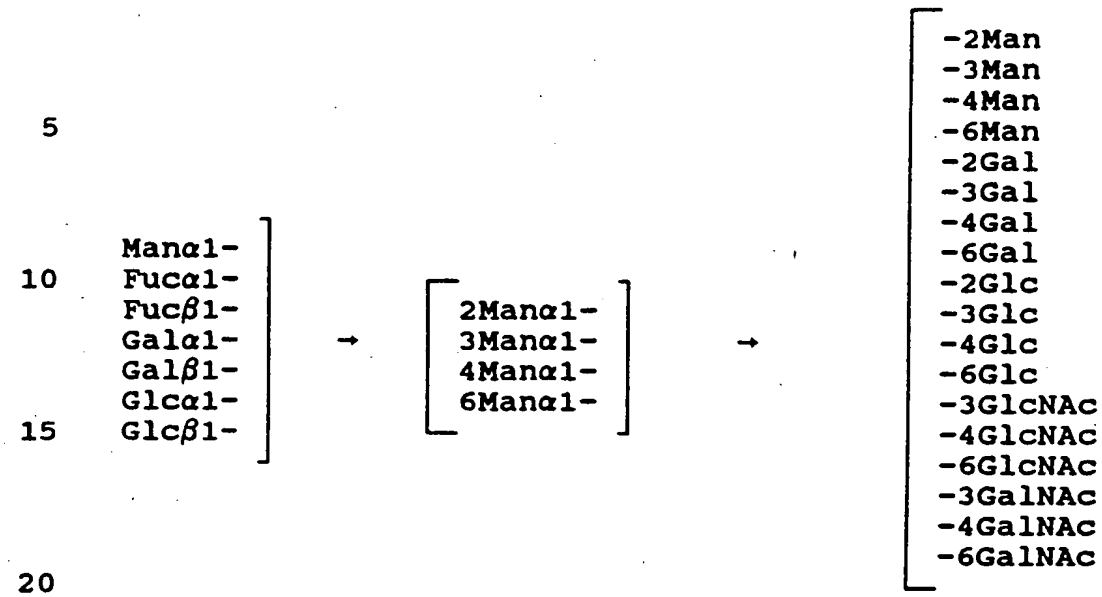
The large group is of sufficient size to dramatically alter the physical properties of the conjugate. For example, if the carbohydrate is conjugated to PEG, then th conjugate will be insoluble in ether, and will crystallise from ethanol. Therefore, if the conjugate is treated with a glycosylation agent, then the unaltered conjugate and the glycosylated conjugate can be separated from excess glycosylation agent and by-products, and the conjugates re-glycosylated.

It is one advantage of the invention that, as som glycosylation reactions do not go to completion, separation of the conjugates from the reaction mixture allows them to be re-glycosylated with fresh glycosylation agents. Further, by having the conjugate linked via a benzyl group, a wide variety of reagents is compatible with the system, while allowing de-conjugation to take place under mild conditions.

Another advantage of the invention, especially with respect to the prior art, is that the precipitatable moiety is coupled to the 1- (or anomeric) position, and via a cleavable linkage. Cleavage is relatively easily performed, e.g. using hydrogenation conditions such as H_2/Pd, yet elaboration of other positions of the sugar ring is possible.

Example

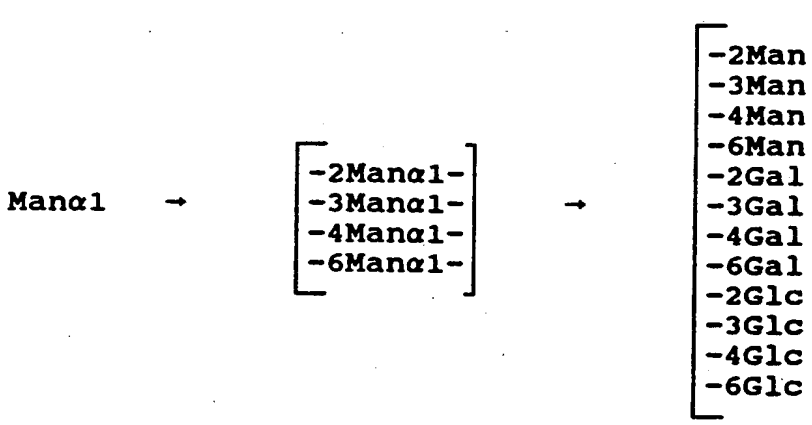
A 504-component linear trisaccharide library with mannose as the central component was constructed:



7 x 4 x 18 = 504 components

In practice the library was constructed as 14 sub-libraries, 7 of which had all 48 trisaccharide components prepared from non-amino sugars and 7 had 24 trisaccharide components where the reducing monosaccharide was an amino sugar.

Construction of the Sub-library



For the preparation of all monosaccharide sub-units, known carbohydrate synthetic methodology was applied. For all glycosylation reactions, the known activating system consisting of N-iodosuccinamide with a catalytic amount of trifluoromethanesulfonic acid was used, all other conditions being well known in the literature. Other glycosylation r action conditions would also be applicable

to this method. For a general review see Seminars in Cell Biology Vol 3. [ed. R.B.Parekh, R.Dwek] (1992).

The following 12 monosaccharide acceptor components were prepared:

1,3,4,6-tetrabenzylmannose
1,2,4,6-tetrabenzylmannose
1,2,3,6-tetrabenzylmannose
1,2,3,4-tetrabenzylmannose
1,3,4,6-tetrabenzylgalactose
1,2,4,6-tetrabenzylgalactose
1,2,4,6-tetrabenzylgalactose
1,2,3,4-tetrabenzylgalactose
1,3,4,6-tetrabenzylglucose
1,2,4,6-tetrabenzylglucose
1,2,3,6-tetrabenzylglucose
1,2,3,4-tetrabenzylglucose

An equimolar mixture of all 12 was made and divided into 4 equal aliquots.

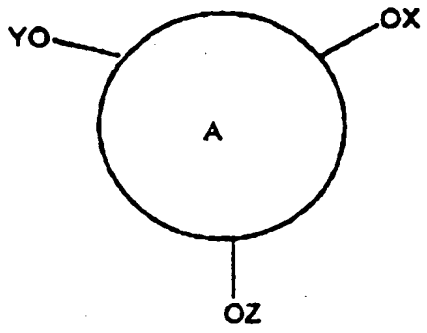
The following 4 differentially-protected monosaccharide donor components were prepared:

methyl 2-acetyl-3,4,6-tribenzyl-1-thio-α-mannose Ia
methyl 3-acetyl-2,4,6-tribenzyl-1-thio-α-mannose Ib
methyl 4-acetyl-2,3,6-tribenzyl-1-thio-α-mannose Ic
methyl 6-acetyl-2,3,4-tribenzyl-1-thio-α-mannose Id

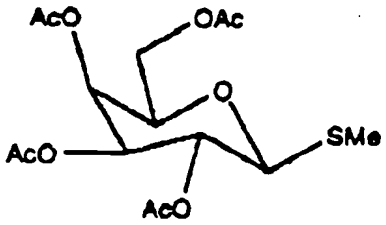
One aliquot of the acceptor components mixture was glycosylated with an excess of Ia, a second aliquot with an excess of Ib and so on, to produce 4 disaccharide mixtures which were then combined, de-O-acetylated by the action of 0.1M sodium methoxide in methanol at room temperature. Excess monosaccharides derived from the donors Ia-Id were removed by means of normal phase silica gel chromatography and the resulting mixture was glycosylated with an excess of methyl 2,3,4,6-tetra-O-acetyl-1-thio-α-mannose to afford the crude trisaccharide library mixture. Following de-O-acetylation as carried out previously, excess monosaccharide was again removed by silica gel chromatography. The protected sub-library was fully deprotected by hydrogenation at RT and air pressure using 10% Pd/C catalyst.

Th sub-library was analysed by P4 gel filtration siz exclusion chromatography and found to contain > 80% of th material as trisaccharide components.

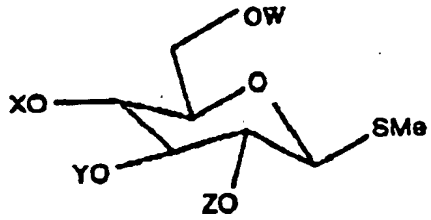
All other sub-libraries were similarly constructed.


YO
OX
A
OZ

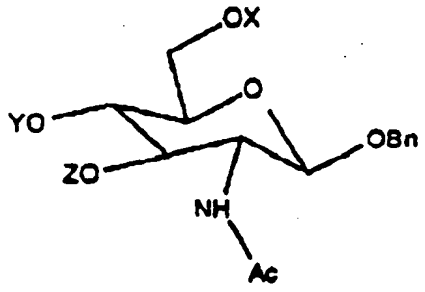
I


AcO
OAc
O
SMe
AcO
AcO

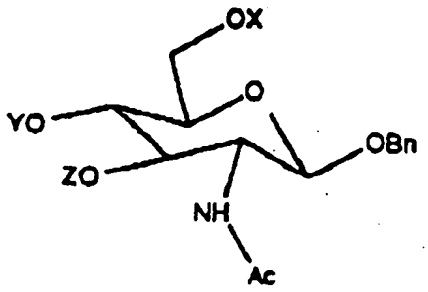
III


OW
O
XO
SMe
YO
ZO

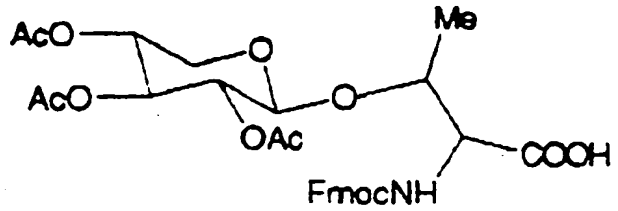
IV


OX
O
YO
OBn
ZO
NH
Ac

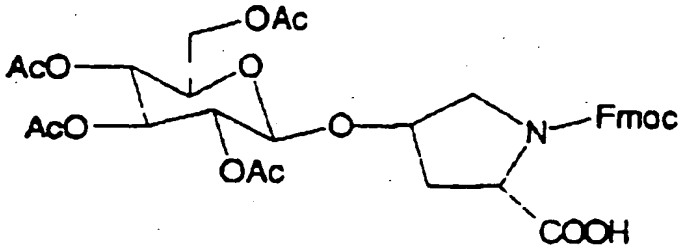
V


OX
O
YO
OBn
ZO
NH
Ac

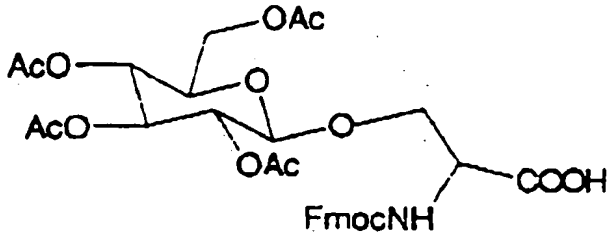
VI



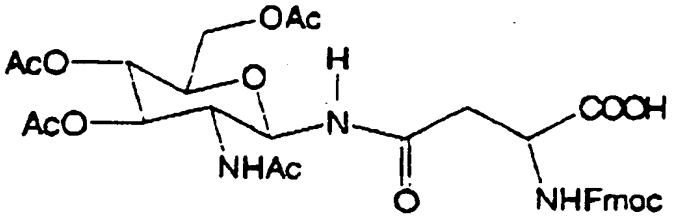
IIA



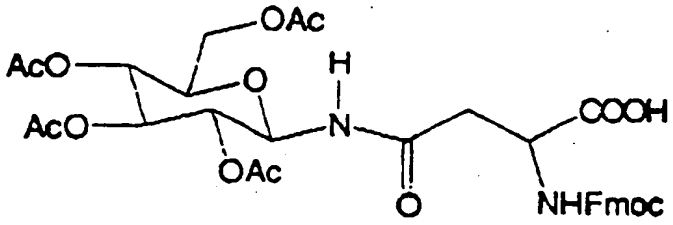
IIB



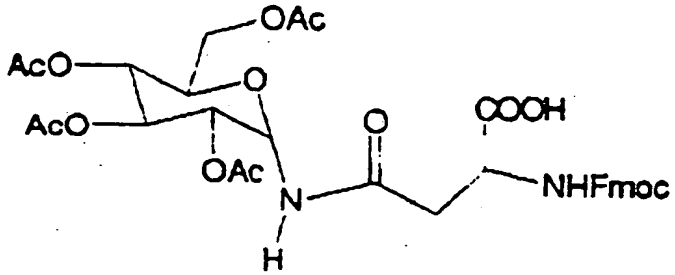
IIC



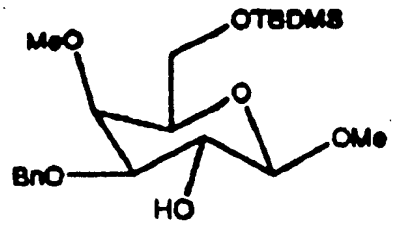
IID



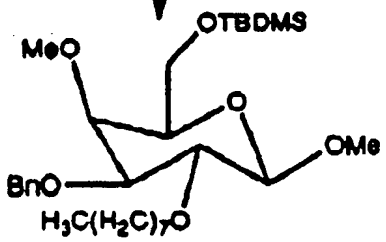
IIE



IIF



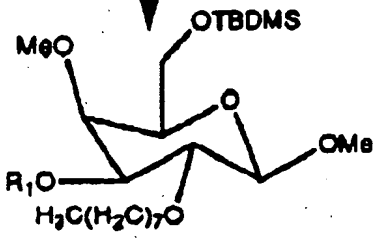
XI



XII

1) H_2/Pd/C

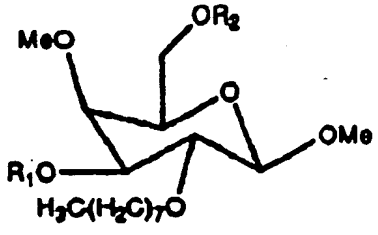
2) R_1Br_1



XIII

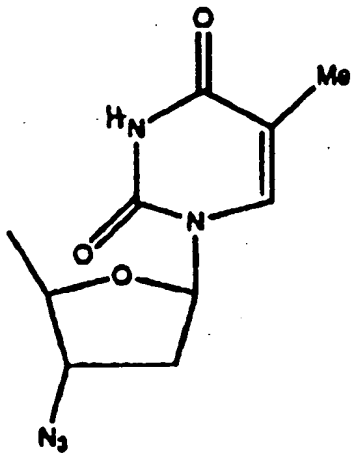
1) CF_3COOH

2) R_2Br_1

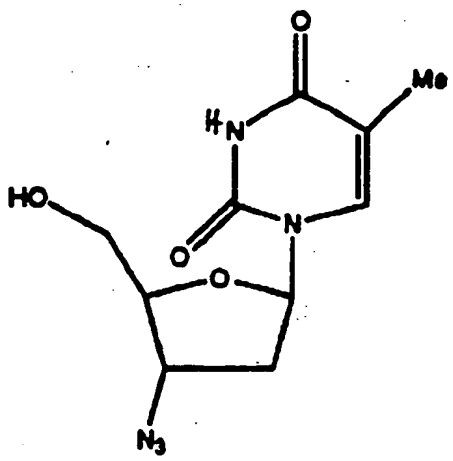


XIV

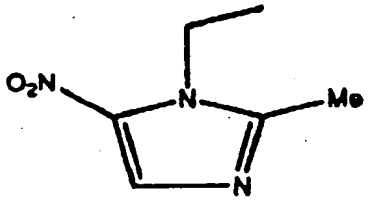
R_1 =



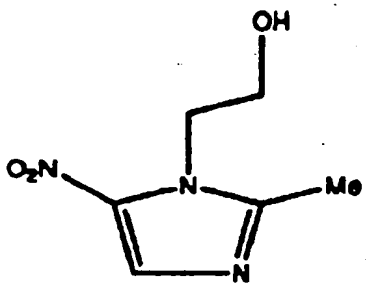
AZT =

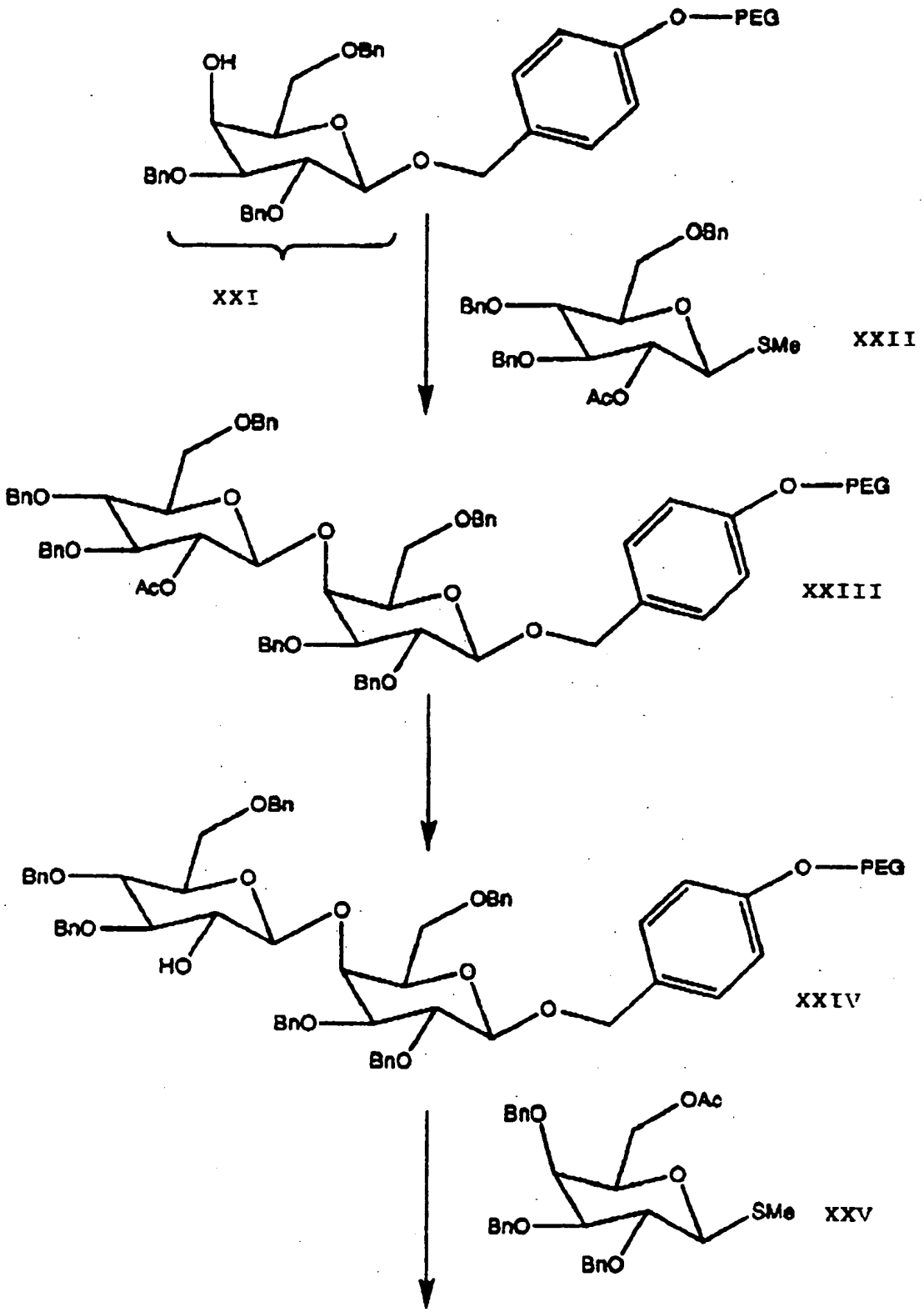


R_2 =



Flagyl =




O—PEG
OH
OBn
O
BnO
O
BnO
XXI
OBn
BnO
O
BnO
SMe
AcO
XXII
OBn
BnO
O
BnO
AcO
O
OBn
O—PEG
O
BnO
O
BnO
XXIII
HO
XXIV
OAc
BnO
O
BnO
SMe
BnO
XXV

CLAIMS

1. A library of at least 6 different sugar-containing molecules selected from (i) carbohydrates each derived from at least 2 saccharide monomers, and (ii) glyc conjugates.

2. A library according to claim 1, wherein the number of different sugar-containing molecules is at least 10.

3. A library according to claim 1, wherein the number of different sugar-containing molecules is at least 20.

4. A library according to claim 1, wherein the number of different sugar-containing molecules is at least 30.

5. A library according to any preceding claim, which comprises substantially equimolar amounts of the different sugar-containing molecules.

6. A library according to any preceding claim, wherein the sugar containing molecules are carbohydrates.

7. A library according to claim 6, obtainable by treating a mixture of monosaccharides with a donor monosaccharid under conjugating conditions, and repeating the treatment one or more times, the saccharide in each step being chosen independently.

8. A library according to claim 6 or claim 7, of disaccharides or linear or branched trisaccharides.

9. A library according to any of claims 1 to 5, wherein the sugar-containing molecules are glycoconjugates.

10. A library according to any of claim 9, wherein the glycoconjugates are glycopeptides.

11. A library according to claim 9 or claim 10, obtainable by treating a mixture of saccharides with a conjugating reagent under conjugating conditions.

12. Use of a library according to any preceding claim, for screening.

13. Use according to claim 12, for screening for activity against a defined therapeutic molecule or activity.

14. A compound having at least 2 OH groups which are modified by independently-removable blocking groups.

15. A comp und according to claim 14, which has at least 3 OH groups and at least 2 of them are modified by independently-removable blocking groups.

16. A compound according to claim 14 or claim 15, which is cyclic.

17. A compound according to claim 16, which is a blocked monosaccharide.

18. A compound according to any of claims 14 to 17, which is chiral (with or without the blocking groups).

19. Use of a compound according to any of claims 14 to 18, for the manufacture of a conjugate in which said compound is bonded to at least two different components.

20. Use according to claim 19, in which the conjugate comprises 2, 3, 4 or 5 different said components.

21. Use according to claim 19 or claim 20, in which said compound is a saccharide and the said different components are not.

22. A conjugate as defined in any of claims 19 to 21, for use in therapy or diagnosis, in which at least one of the different components has therapeutic or diagnostic utility.

23. Use of a conjugate of claim 22, for the manufacture of a medicament for use in therapy associated with the or each utility.

24. A library of conjugates as defined in any of claims 19 to 21.

25. A library according to claim 24, which comprises substantially equimolar amounts of the conjugates.

26. A process for preparing a library according to claim 24 or claim 25, which comprises the steps of:

reacting a plurality of samples of a mixture of compounds according to any of claims 14 to 18 with a respective plurality of reagents that replace a blocking group or react with a free OH group;

removing another blocking group; and

repeating the preceding steps, starting with the products of the previous step, one or more times, as desired.

27. A process for synthesising a polysaccharide of the formula $sugar_n$, n representing the number of mono- and/or oligo-saccharide units from which the polysaccharide may be derived, which comprises the steps of coupling $sugar_2$ with a conjugate of the formula Q-$sugar_1$, Q being a removable

lipophilic or polymeric material, in a solvent for th conjugate; adding a non-solvent for the resultant conjugate Q-sugar$_1$-sugar$_2$; repeating the said steps n-2 times, as necessary, sel cting the sugar to be coupled independently each time; and removing Q from the resultant coupled conjugate.

28. A process according to claim 27, wherein sugar$_1$ is conjugated to Q at the anomeric position.

29. A process according to claim 27 or claim 28, wherein Q is modified PEG.

30. A process according to any of claims 27 to 29, wherein sugar$_1$ is coupled via a benzyl group.

31. A process according to any of claims 29 to 30, wherein ether is the non-solvent.

32. A process according to any of claims 27 to 31, wherein any excess sugar$_2$, sugar$_3$ etc. and any activator is removed before each repetition of the said steps.

33. A process according to claim 32, wherein one or more of sugar$_2$, sugar$_3$ etc. is soluble in the solvent.